GeneTrace Systems, Inc.

Development of Rapid DNA Medical Diagnostics

In the early 1990s, researchers were looking into new forms of medical treatment and diagnosis that had become available through emerging technologies. In particular, DNA analysis could explore the potential weaknesses of diseased cells and interactions between them. However, the analytical process in use at the time, gel-based electrophoresis separation, was slow and costly and required lengthy data analysis. Two researchers from SRI International formed GeneTrace Systems, Inc. and applied to the Advanced Technology Program (ATP) for support in automating DNA analysis. In January 1995, ATP awarded GeneTrace cost-shared funding through the 1994 "Tools for DNA Diagnostics" focused competition. If successful, the technology could alter the treatment of cancer and other diseases by rapidly developing gene libraries that could substantially shorten drug development.

During the ATP-funded project, GeneTrace developed a mass spectrometry system that could sequence thousands of DNA bases per day using an automated, robotic process, compared to only two dozen per day using gel-based electrophoresis. The company licensed this service to other biotechnology companies and packaged the system for sale to companies that preferred to perform the service themselves. GeneTrace also manufactured sample arrays, which contained the system's chemical solutions, for DNA testing. The company received patents for its technology and shared its research through publications and presentations. The body of knowledge created by GeneTrace significantly contributed to the understanding of genome analysis within the biotechnology industry. Although GeneTrace is no longer in business, two companies, Sequenom and Althea Technologies, acquired most of its assets. Althea Technologies is using the gene expression technology in disease modeling, target discovery and validation, and agricultural screening, typing, and breeding.

COMPOSITE PERFORMANCE SCORE
(based on a four star rating)
* * *

Research and data for Status Report 94-05-0006 were collected during September – October 2004.

Rising Healthcare Costs Prompt Search for Treatment Technologies

In the early 1990s, new forms of diagnosis and treatment were evolving to combat cancer, AIDS, cardiovascular disease, viral infections, and inherited genetic disorders such as amyotrophic lateral sclerosis and muscular dystrophy. Analysis of genetic code by DNA sequencing was yielding fundamental knowledge that was increasingly used in drug design and medical testing. Such sequencing was the standard technique for determining cellular codes, or the precise order in which four chemical bases appear in a strand of genetic material. The most common form of DNA sequencing was gel-based electrophoresis separation, a slow and costly method that entailed tedious data analysis to interpret the results.

Two researchers from SRI International (Palo Alto, CA) founded GeneTrace Systems, Inc. to develop a highly automated mass spectrometry system. If successful, this system would be capable of rapid and cost-effective DNA tests for its primary targets of infectious disease organisms and hereditary

genetic disease. They faced three major technical challenges:

- Chemistry for coupling and decoupling the primers, or short sequences of DNA used in polymerase chain reactions, was not fast or efficient enough for commercial use. The company thought it could modify existing technology by introducing a sample array.
- The error rate of mass spectrometry determinations needed to be one percent or lower, while keeping the rate of data transmission high.
- Controlling the movement of small volumes of liquid would be difficult.

The company applied for funding from ATP under the 1994 "Tools for DNA Diagnostics" focused program. In January 1995, ATP awarded GeneTrace cost-shared funds for a three-year project to develop an automated, rapid means for DNA sequencing for clinical diagnostics and biomedical research.

The most common form of DNA sequencing was gel-based electrophoresis separation, a slow and costly method.

Time-of-flight mass spectrometry (TOF-MS) was a new, extremely fast way of analyzing DNA molecules using pulsed laser light to propel molecules through the instrument, which separates them by size. Using computers, mass spectrometry instrument systems can detect and identify DNA molecules that differ by a single base. GeneTrace predicted that its technology would perform 100 times faster than commercial state-of-the-art DNA sequencing and would reduce costs dramatically.

Spectrometer Promises Commercial Prospects

GeneTrace intended to develop automated equipment for a fully working prototype by the end of the second year of the project. They would then seek the Food and Drug Administration's approval of a diagnostic test for hospitals and clinical laboratories. The company's long-term objective was to manufacture diagnostic equipment and supplies as well as to expand to screening tests for predisposition to cancer, forensic tests, and identification tests such as paternity tests. The company would develop service laboratories to conduct the tests. GeneTrace proposed that the same TOF-MS device could perform all of these tests by changing the arrays.

Bacterial blood culture testing alone accounted for approximately 10 million tests performed in the United States in 1993. These tests were used extensively among immune-compromised patients, such as chemotherapy, organ transplant, and AIDS patients. The testing technology took one to two days to provide results, a clinically undesirable response time for raging systemic infections. After the culture, an array of further tests could be warranted, depending on the circumstances. Overall false-negative rates for the tests were often high (5 to 30 percent).

The GeneTrace test would simplify the diagnostics, shorten the response time to a few hours, and reduce error rates, at a lower per-test cost. The test could be used on viruses such as HIV and hepatitis B as well as bacteria. It was important to find the mechanism that makes a bacterium resistant to antibiotics, so that targeted drugs could be developed.

GeneTrace Pursues Aggressive Strategy

GeneTrace continued to seek funds for clinical trials of a generalized infectious disease test (capable of determining the presence of up to 50 diseases) and of a hereditary genetic disease test. The company expected to develop applications for specific cancer-related diagnostics and patient-specific gene therapy agents or medicines.

As the first year of the project came to an end, GeneTrace had built and operated a laboratory TOF-MS device that sequenced DNA using proprietary formulas. The technology combined sequencing reactions with TOF-MS to yield rapid molecular sizing of the chemical reaction's products. The TOF-MS's first run analyzed 50 different infectious organisms, including antibiotic-resistant strains. Unexpectedly, the company also developed a mass-resolution-enhancing technique that improved the device's resolution and

signal-to-noise ratios, making the device much more accurate and efficient in analyzing DNA.

The company also began an informal relationship with Incyte Pharmaceuticals, an industry leader in the design, development, and marketing of genomic database products and services. In addition to its relationship with Incyte, GeneTrace formed business partnerships with SRI International, MJ Research Inc., CyberLab, Amersham, and Pharmacia.

GeneTrace increased the TOF-MS's capacity to sequence longer bases much faster than the conventional method, with nearly the same results. The company built an automated laboratory mass spectrometer system machine that could analyze an array of samples at the rate of 3 seconds per reaction, compared to the traditional 10 minutes by hand. This achievement brought the company closer to its goal of manufacturing commercial-scale TOF-MS devices for sale to biotechnology and pharmaceutical industries. The company applied for four patents: two for oligonucleotide sizing; one for a mass spectrometer; and one for methods of preparing and screening nucleic acids for mass spectrometric analysis.

Second Year Yields Industry Agreement

In the second year of the ATP-funded project, GeneTrace developed molecular biology tools that were tailored to their analytical platform. This allowed researchers to study the sequencing of DNA as it appears in practical diagnostic problems. The company also completed two additional automated TOF-MS systems and continued to develop assays for gene analysis.

In late March 1996, GeneTrace signed a contract with Incyte Pharmaceuticals in which Incyte would license GeneTrace's mass spectrometry technology and collaborate in developing specific DNA analysis applications. In addition to acquiring a minority stake in the company, Incyte agreed to support GeneTrace research and development (R&D) with milestone payments and technology license fees. GeneTrace received two milestone payments for performing highly accurate sequencing of a large number of DNA samples using its automated software.

Marian Marra, Director of R&D Ventures for Incyte, said, "Mass spectrometry DNA analysis is the first in a series of technology investments which we hope will create a highly efficient, integrated process that encompasses everything from DNA sample preparation to the functional analysis of genes. These technologies should allow Incyte to rapidly screen thousands of genes at a time, to study gene function in multiple biological samples."

Sequana Therapeutics Inc., Berlex/Schering AG, Algene Inc., Varian Inc., and the National Institutes of Health Center for Inherited Disease Research all partnered with GeneTrace to develop new sequencing projects. D2M Inc. supplied designs for the manufacturing prototype of the TOF-MS and associated electronics. Surface/Interface Inc. helped to refine the sample input design for the TOF-MS system.

GeneTrace also formed several academic collaborations. In genotyping, the company worked with Stanford University on typing single nucleotide polymorphisms (SNPs), or variations in a sequence. SNPs can affect how humans handle diseases, bacteria, viruses, chemicals, or drugs. GeneTrace collaborated with the University of Washington on SNP automation and human forensics.

Time-of-flight mass spectrometry was a new, extremely fast way of analyzing DNA molecules using pulsed laser light to propel molecules.

GeneTrace had applied for two more patents by the end of 1997: for methods of preparing screening nucleic acids using mass spectrometry and for nonvolatile mass label molecules.

Project End Brings Technical and Commercial Achievements

By the conclusion of the ATP-funded project in 1997, GeneTrace Systems had grown from 5 employees to 15. In addition to its two laboratory mass spectrometer systems for in-house laboratory use, the company had made prototype commercial-scale mass spectrometers. With these machines, GeneTrace could run more than 250 sequences per hour per instrument and 480 assays

per hour of SNPs, which are used in biomedical research and pharmaceutical laboratories. The company could also run 400 assays per hour of microsatellites (useful for genetic fingerprinting and paternity testing) or short, noncoding sequences (DNA without a code for making proteins or other cell products).

GeneTrace continued to sequence cancer cell clone libraries and to analyze SNPs, In addition, GeneTrace had been awarded a patent for oligonucleotide sizing, had published four articles, and had made eight presentations.

After the project ended, these technical achievements brought commercial success and attention from agricultural, in vitro diagnostics, and forensics industries. Based on business relationships developed during the project, in 1998 GeneTrace began collaborating with Monsanto Company in plant and animal genomics research. The agreement included options to license all aspects of GeneTrace's genomics technologies worldwide and a $17.2 million equity investment in GeneTrace. Monsanto sought to use GeneTrace's TOF-MS system to analyze genes from corn, soybeans, and other crop plants. GeneTrace applied for three patents in 1998 for mass spectrometry sample processing, methods of preparing nucleic acids for mass spectrometry, and DNA typing by mass spectrometry.

Technical achievements brought commercial success and attention from agricultural, in vitro diagnostics, and forensics industries.

In addition, GeneTrace had achieved the following:

- Signed an agreement with Berlex Laboratories Inc. in November 1999 to apply GeneTrace's DNA analysis to study several of Berlex's human cancer gene samples.
- In March 2000, acquired Strata Biosciences, which was pioneering methods for gene discovery, drug target identification, and validation. These methods widened GeneTrace's study of the complex nature of biological systems in their entirety.
- Reached an agreement with Molecular Mining Corporation in January 2001 to co-fund research and share revenues from collaborative design, analysis, validation, nature, and definition of physiological models for application to drug discovery.
- Applied for three patents in 2000 and 2001 for screening methodology, DNA typing, and analysis of gene expression.

Despite its technical and commercial success, after the stock market downturn and some of the company's corporate partners made strategic business changes, GeneTrace Systems went out of business. The company had contributed significantly to the industry's knowledge base, and all 12 patents based on the company's technology have since been issued. In late 2001, Sequenom, a larger genomics company that also used mass spectrometry, acquired much of GeneTrace's intellectual property. Sequenom may use GeneTrace's TOF-MS technology in DNA sequencing and SNP identification. GeneTrace sold its remaining technology to Althea Technologies, which is using GeneTrace's gene expression technology in dozens of commercial studies on toxicological mechanisms, drug pharmacodynamics in animals and humans, in vitro drug studies, and plant genetic screens. In March 2004, Althea licensed its GeneTrace-based gene expression technology to Beckman Coulter, Inc. which launched it as kits, reagents, software, and equipment in its GeXP product line.

Conclusion

GeneTrace Systems entered into biotechnology research and development (R&D) when DNA testing for infectious and hereditary diseases was promising but still had significant obstacles. GeneTrace carried out several R&D tasks simultaneously and improved on the basic technology throughout the project, even after it had begun to achieve commercial success. The company's breakthrough technology was in linking time-of-flight mass spectrometry with highly efficient DNA sequencing and developing a system that sped up analysis. The system could separate and detect DNA strands at a rate of 1,000 samples per hour per instrument, a much higher output than conventional gel electrophoresis and analysis.

The company focused on genetic profiling of compound libraries, selection of new drug leads, and identification and ranking for new drug targets. GeneTrace developed academic relationships with Stanford University and the University of Washington and pursued business relationships, beginning with a pharmaceutical company and branching out to the agricultural, in vitro diagnostics, and forensics industries. GeneTrace's technical success and subsequent diffusion of research within the biotechnology industry would not have been possible without the financial backing of ATP. GeneTrace was awarded patents for its technology and shared its research through publications and presentations. Although the company went out of business in 2001, its technology was acquired by two other companies, Sequenom and Althea Technologies. Althea is using GeneTrace's technologies in its product lines and has licensed some of these technologies to another company. In the future, Sequenom may use the time-of-flight mass spectrometry technology in DNA sequencing.

PROJECT HIGHLIGHTS
GeneTrace Systems, Inc.

Project Title: Development of Rapid DNA Medical Diagnostics

Project: To develop an automated, rapid means for fully determining DNA sequencing, primarily for clinical diagnostics and biomedical applications.

Duration: 1/1/1995–12/31/1997
ATP Number: 94-05-0006

Funding (in thousands):

ATP Final Cost	$1,997	74%
Participant Final Cost	699	26%
Total	$2,696	

Accomplishments: With ATP funding, GeneTrace Systems, Inc. accomplished the following:

- Developed a fully automated DNA sequencing system that can conduct an analysis hundreds of times faster than gel-based methods at a fraction of the cost
- Developed software to make accurate distinctions among the various components of DNA

GeneTrace Systems, Inc. also received the following patents for technologies related to the ATP-funded project:

- "Oligonucleotide sizing using immobilized cleavable primers"
  (No. 5,700,642: filed May 22, 1995, granted December 23, 1997)
- "Oligonucleotide sizing using cleavable primers"
  (No. 5,830,655: filed April 26, 1996, granted November 3, 1998)
- "Mass spectrometer"
  (No. 5,864,137: filed October 1, 1996, granted January 26, 1999)
- "Methods of preparing nucleic acids for mass spectrometric analysis"
  (No. 5,965,363: filed December 2, 1996, granted October 12, 1999)
- "Methods of screening nucleic acids using mass spectrometry"
  (No. 6,051,378: filed March 4, 1997, granted April 18, 2000)
- "Releasable nonvolatile mass label molecules"
  (No. 6,635,452: filed December 10, 1997, granted October 21, 2003)
- "Volatile matrices for matrix-assisted laser desorption/ionization mass spectrometry"
  (No. 6,104,028: filed May 29, 1998, granted August 15, 2000)
- "Methods of preparing nucleic acids for mass spectrometric analysis"
  (No. 6,566,055: filed June 3, 1998, granted May 20, 2003)
- "DNA typing by mass spectrometry with polymorphic DNA repeat markers"
  (No. 6,090,558: filed September 18, 1998, granted July 18, 2000)
- "Methods of screening nucleic acids using volatile salts in mass spectrometry"
  (No. 6,468,748: filed February 29, 2000, granted October 22, 2002)
- "DNA typing by mass spectrometry with polymorphic DNA repeat markers"
  (No. 6,764,822: filed April 3, 2000, granted July 20, 2004)
- "Methods for analysis of gene expression"
  (No. 6,618,679: filed January 27, 2001, granted September 9, 2003)

Commercialization Status: After GeneTrace was dissolved, two companies purchased its technology. Sequenom may use the time-of-flight mass spectrometry technology in DNA sequencing. Althea Technologies is using GeneTrace's gene expression technology for disease modeling, target discovery and validation, toxicological screening, animal typing, and agricultural breeding.

Outlook: The technology developed under this project appears to be versatile and highly useful to genomics. Time-of-flight mass spectrometry is used in DNA sequencing in medical and security applications; gene expression technology is used for medical modeling and in agricultural applications. The outlook for this technology is strong.

Composite Performance Score: * * *

Number of Employees: 5 employees at project start, 0 as of January 2005.

PROJECT HIGHLIGHTS
GeneTrace Systems, Inc.

Focused Program: Tools for DNA Diagnostics, 1994

Company:
GeneTrace Systems is no longer in existence

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Publications:

- Li, J., et al. "Genetic Analysis of Short Tandem Repeat Loci by Time-Of-Flight Mass Spectrometry." *Proceedings of the Seventh International Symposium on Human Identification*, ed. J.W. Schuum, pp.158-162, 1996.
- Shaler, T., et al. "Effects of Impurities on the Matrix-Assisted Laser Desorption Mass Spectra of Single-Stranded Oligonucleotides." *Anal. Chem.*, vol. 68, pp. 576-579, 1996.
- Butler, J.M., et al. "Rapid and Automated Analysis of Short Tandem Repeat Loci Using Time-Of-Flight Mass Spectrometry." *Proceedings of the Eighth International Symposium on Human Identification*, ed. J.W. Schuum, 1997.
- Monforte, J. and C.H. Becker. "High Throughput DNA Analysis by Time-Of-Flight Mass Spectrometry." *Nature Medicine*, vol. 3, pp.360-362, 1997.
- Butler, J.M. and C.H. Becker. "Improved Analysis of DNA Short Tandem Repeats With Time-Of-Flight Mass Spectrometry." *Science and Technology Research Report*, U.S. Department of Justice, Office of Justice Programs, National Institute of Justice, Washington, D.C., October 2001.

Presentations:

- Cambridge Healthtech Institute (CHI) Conference on Genomics, San Francisco, CA, February 24–26, 1997.
- International Business Communications (IBC) Conference on Genomics, San Diego, CA, March 5–6, 1997.
- IBC Conference on Gene Mutational Analysis, New Orleans, LA, May 19–20, 1997.
- American Society for Mass Spectrometry Conference, Palm Springs, FL, June 4, 1997.
- CHI Conference on Advances in Labels, Signaling & Detection, San Diego, CA, June 5–6, 1997.
- IBC Conference on Functional Genomics, San Diego, CA, September 25–26, 1997.
- IBC Conference on Biochip Technologies, Annapolis, MD, October 6–8, 1997.
- IBC Conference on Genetic Profiling & Diagnostics, San Diego, CA, October 29–30, 1997.